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Oxygen, evolution and redox signalling in the human brain; quantum in the quotidian

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Abstract

Rising atmospheric oxygen (O_2) levels provided a selective pressure for the evolution of O_2-dependent micro-organisms that began with the autotrophic eukaryotes. Since these primordial times, the respiring mammalian cell has become entirely dependent on the constancy of electron flow with molecular O_2 serving as the terminal electron acceptor in mitochondrial oxidative phosphorylation. Indeed, the ability to "sense" O_2 and maintain homeostasis is considered one of the most important roles of the central nervous system (CNS) and likely represented a major driving force in the evolution of the human brain. Today, modern humans have evolved with an oversized brain committed to a continually active state and as a consequence, paradoxically vulnerable to failure if the O_2 supply is interrupted. However, our pre-occupation with O_2, the elixir of life, obscures the fact that it is a gas with a Janus Face, capable of sustaining life in physiologically controlled amounts yet paradoxically deadly to the CNS when in excess. A closer look at its quantum structure reveals precisely why; the triplet ground state diatomic O_2 molecule is paramagnetic and exists in air as a free radical, constrained from reacting aggressively with the brain's organic molecules due to its "spin restriction", a thermodynamic quirk of evolutionary fate. By further exploring O_2's free radical "quantum quirkiness" including emergent (quantum) physiological phenomena, our understanding of precisely how the human brain senses O_2 deprivation (hypoxia) and the elaborate redox-signalling defence mechanisms that defend O_2 homeostasis has the potential to offer unique insights into the pathophysiology and treatment of human brain disease.

Astronomers recently detected faint signals of ancient ionized oxygen (O_2), the most distant ever discovered, emitted a staggering 13.28 billion years ago (Gya), indicating that stars began forming just 500 million years after the Big Bang when the universe was less than 4 % its current age (Hashimoto *et al.*, 2018). Thus, we can thank our (dying) lucky stars, the burning crucibles that convert hydrogen and helium into heavier elements for our O_2, the molecule that made our world, our brains and us. As an element, oxygen (O) is unique; it is the third most abundant element in the universe after hydrogen and helium, the second most electronegative element behind fluorine making it an ideal electron acceptor and the most abundant element in the Earth's crust (Allred & Rochow, 1958; Dole, 1965). However, while free O_2 in the atmosphere distinguishes our planet from all others in the solar system, the early terrestrial atmosphere was not quite so unique.

Coupled evolution of life and O_2

The composition of the ancient atmosphere was largely dictated by volcanic gases and consisted mainly of hydrogen, carbon dioxide, carbon monoxide, hydrogen sulfide and methane (Holland, 2002). Given the ubiquity of the proton gradient in cells, life likely emerged in alkaline thermal vents at the bottom of the oceans, eventually giving rise to two orders of life, archaea and bacteria (Miller & Bada, 1988). However, it wasn't until ~1.5 Gya that photosynthesising blue-green algae (cyanobacteria) began to breathe life into what was effectively a reductive, anaerobic atmosphere splitting water to obtain the hydrogen required to drive metabolic reactions ($2H_2O \rightarrow 4H + O_2\uparrow$)(Nisbet & Sleep, 2001). The inexorable rise in atmospheric O_2 during the Proterozoic Eon of the Pre-Cambrian period ~2,500-540 million years ago (Myr) signaled a death sentence to anaerobes yet

sparked an explosion of the planet's biota and saw the number and diversity of multicellular species expand exponentially (Berner *et al.*, 2007).

Figure 1A illustrates the major evolutionary and developmental events that have been inextricably linked to atmospheric O_2 "pulses" over two oxidation events, the Great Oxidation Event (GOE) and Neoproterozoic Event (NOE) interspersed by the Boring Billion, a period between ~1.8 and 0.8 Gyr ago in Earth's history characterised by geobiological stasis (unchanging pattern of atomic isotopes and redox states) characterised by unremarkably stable atmospheric and oceanic O_2 levels (Holland, 2006). This lack of O_2 has been linked to a delay in the evolution of complex life (Lyons et al., 2014) though this has recently been contested given that numerous critical biological evolutionary events, such as the appearance of eukaryotes, origin of multicellularity and sexual reproduction and the first major diversification of eukaryotes occurred during this period, triggering evolutionary pathways that facilitated the later rise of micro-metazoans and their macroscopic counterparts (Mukherjee et al., 2018). Furthermore, the two-step transition from a virtually anoxic environment to present day conditions has been challenged by a more gradual increase in O_2 levels, termed the Great Oxidation Transition (Lyons *et al.*, 2014). Likewise, though beyond the remit of the current review, other atmospheric gases, notably carbon dioxide, has also helped shape life on Earth to which the brain has evolved heightened sensitivity buffering brain tissue pH for stabilisation of chemosensory and autonomic control at the level of the brainstem (Cummins *et al.*, 2014; Willie *et al.*, 2014; Bailey *et al.*, 2017b).

Being surrounded by O_2 likely favored the survival of organisms capable of tolerating the toxicity associated with its damaging free radical reactions (see later), specializing in cellular mechanisms that could harness the gas safely to generate energy giving rise to aerobic respiration, central to oxidative phosphorylation and bioenergetic homeostasis following a symbiotic merger with the once

free-living *α*-proteobacteria that subsequently gave way to the more sophisticated energy powerhouse, the mitochondrion (Gray *et al.*, 2001). Chemical reduction by the mitochondrial electron transport chain has since seen O_2 become the (ideal) terminal electron acceptor reducing it to water, its thermodynamic "nirvana", supplying ~30 molecules of adenosine triphosphate (ATP) per metabolized glucose molecule to the respiring eukaryote. This provided efficient, regulated metabolic support signaling the development of more complex structures such as the early brain in bilateri, conferring a clear evolutionary advantage over the 2 ATP/glucose yield by the more basic anaerobic glycolytic reaction.

The inextricable link between O_2 and biological evolution is especially evident over the more recent Phanerozoic Eon (~550 Myr) when atmospheric levels increased to between 15-20% sparking the first animal body plans marking the advent of metazoan evolution (Figure 1B) (Berner *et al.*, 2007). Further elaborations to O_2 transport systems included the emergence of the parallel pulmonary circulation and the four chambered heart during the Permian when atmospheric O_2 levels peaked during the late Carboniferous period reaching a staggering 35 %, imposing fewer limits on O_2 diffusion allowing the giant Carboniferous dragonfly (*Meganeura monyi*) with a wing span in excess of 75 cm to flourish (Graham *et al.*, 1995). It would seem intuitive that further refinements were made to endogenous antioxidant defences to cope with this extra O_2 (Halliwell, 2006); indeed, some of the plants that evolved at that time are more O_2 resistant than more recently evolved plants (Beerling *et al.*, 1998).

Notwithstanding the finer details, contemporary estimates now suggest that the green plants on earth combine a total of 150 billion tons of carbon (from CO_2) with 25 billion tons of H_2 (from H_2O) to liberate 400 billion tons of O_2 each year to maintain O_2 at its current atmospheric level (Bailey, 2001). However, it is unlikely that O_2 is here to stay since there has been an inexorable decline in

atmospheric levels over the past 20 years. Originally assumed to be linear (equivalent to ~4 ppm/year), more recent estimates suggest that the decline is more likely parabolic (Livina *et al.*, 2015). Application of this parabolic projection to original data (Keeling, 1988) makes for some startling if not indeed catastrophic predictions (Martin *et al.*, 2017) as outlined in Figure 1C notwithstanding the predictive constraints associated with a mathematical (as opposed to a geochemical) model. Within ~3,600 years from now, it is predicted that atmospheric O_2 levels will become so low that even living at sea-level will feel as hypoxic as living at an equivalent terrestrial altitude of ~5,340 m, the highest elevation known to sustain lifelong human habitation with complete depletion predicted within ~4.4 millennia (Martin *et al.*, 2017). Global deoxygenation may impact brain morphology and hemodynamic function as humans are likely to undergo further selection for physiological phenotypes that confer improved ability to survive chronic hypoxemic stress, potentially resembling those of well-adapted high-altitude populations like the Tibetans and Sherpa (Gilbert-Kawai *et al.*, 2014).

Evolution of the human brain; size and flow mattered

Environmental pressures caused by climatic fluctuations have long been assumed to play a key role in hominin speciation and adaptation (Maslin & Christensen, 2007). Not surprisingly, O_2 has played an especially important role in the development of the human brain, arguably the most significant event in the evolution of human life. The fossil record and neuroanatomical analysis of closely related species indicates that the hominin brain increased in size by ~3.5 fold over a period of ~3 million years (from 400-600 cm^3 to 1,200-1,600 cm^3) with a neocortex that has come to constitute 80% of the brain with disproportionate increases observed in the prefrontal and posterior parietal

cortex (Figure 2A) (Semendeferi *et al.*, 2002; Schoenemann, 2006; Azevedo *et al.*, 2009). With an encephalization quotient of 7 (seven times larger in relation to our expected brain-to-body mass ratio) the modern human is the most encephalised of all species (Hadjistassou *et al.*, 2015).

Furthermore, recent estimates indicate that unlike primates, the increase in human brain volume was accompanied by an even greater (6-fold) increase in global cerebral blood flow to support rapid development in interneuron connectivity, synaptic activity and cognitive function (Seymour *et al.*, 2016). It would thus seem that we ultimately got smarter through a rush of blood to the head! Thus, the brain did not simply become bigger, but more specialized areas were likely added, providing new functions for more complex analysis including cognitive specialisation (Weaver, 2005).

Selection acting on physical endurance capacity and subsequent increases in cerebral perfusion and O_2 delivery may have been the primordial stimulus for accelerated neurotrophin and growth factor signalling that may have contributed to overall brain growth and development as early as 1.8 Myr when our ancestors in particular *Homo Erectus* began walking and running longer distances than previous hominin taxa (Raichlen & Polk, 2013). Given that brain tissue is metabolically expensive (see below), such disproportional increases in brain volume would not likely have occurred unless they conferred some sort of adaptive (reproductive, social, cognitive and ecological) advantages though the finer details remain unresolved.

Cerebral bioenergetics and vulnerability to failure

Today, the “modern” human brain exemplifies our reliance on O_2 because, unlike most other organs, this evolutionary “drive for size” has meant that it is now committed to a continually active state and is entirely aerobic since it does not store glucose or much glycogen constrained by a relatively low

capillary density and thus relies on a constant blood supply (Bailey, 2016; Bailey *et al.*, 2017b). Though it weighs a meagre 2% of our total body mass, the human brain allocates a disproportionate 20-25% of total resting metabolic rate to brain function (Attwell *et al.*, 2010) compared with 8–10% for non-human primates and 3–5% for most non-primate mammals (Leonard *et al.*, 2003). Assuming an average brain mass of 1.4 kg, O_2 is consumed at a rate of ~1.5 mmol/min/g tissue or ~3 mol of O_2/day, generating a staggering ~18 mol or ~9 kg of ATP/day (Figure 2B). To put this into clearer perspective, this is roughly equivalent to what a human leg muscle would generate during a marathon (Attwell & Laughlin, 2001).

This equates to more than 10 times that expected from its mass alone helping power its ~86 billion neurons (Herculano-Houzel, 2012) and complex connectome spanning up to 10^{15} synapses with over 100,000 km of interconnections and ~250–300 billion glia capable of storing anywhere between 58-580 terabytes of information (Nunn *et al.*, 2016). This obligatory requirement to process large amounts of O_2 over a relatively small tissue mass is required to support the high rate of ATP formation to fuel the maintenance of ionic equilibria and uptake of neurotransmitters for synaptic transmission with 40–60% of this energy directed towards moving ions “uphill” with the majority of energy supplied by mitochondria and consumed at the synapses (Alle *et al.*, 2009; Harris *et al.*, 2012). This is even more paradoxical when one considers that lineages with large brains generally exhibit poor hypoxia tolerance, hence one would have expected O_2 constraints to have constrained the evolution of large brain size (Sukhum *et al.*, 2016) and indeed average endocranial volume has decreased by 240 mL during the Holocene (past 10,000 years), ~36 times the rate of increase observed during the previous 800,000 years (Henneberg, 1988).

However, this obligatory high rate of O_2 consumption is associated with high “vulnerability for failure” given the brain’s paradoxically limited O_2 reserves. Assuming an average cerebral tissue

partial pressure of O_2 (PcO_2) of ~25mmHg and lack of O_2-binding proteins, the brain's O_2 content is a meagre ~30 nmoL/mL such that given an average cerebral metabolic rate of oxygen ($CMRO_2$) of 30 nmoL/mL/s, the O_2 present would sustain metabolism for at best 1 second if blood supply were to be interrupted by anoxia (Leithner & Royl, 2014) (Figure 2B). Unable to compromise on its excessive energy budget, failure of ATP-dependent ion exchangers results in the breakdown of ionic gradients and membrane depolarisation triggering a cytotoxic increase in intracellular Ca^{2+} concentration and uncontrolled release of excitatory neurotransmitters that ultimately converge in neuronal death (Lipton, 1999). This can result in devastating consequences, as the clinical complications associated with stroke and head trauma stand testament to.

The paradox of O_2; quandry of quantum quirkiness

Despite it's early appearance, the discovery of O_2 described as "the most important discovery in the history of science" had to wait until 1774 when Joseph Priestley (1733-1804) first described the existence of "dephlogisticated air" by heating mercuric oxide though this remains a hotly contested topic given that the gas had been purified and used to sustain human life and exercise by both a Polish alchemist (Michał Sędziwój, 1566–1636) and Dutch engineer (Cornelis Jacobszoon Drebbel, 1572-1633) some two centuries earlier. Priestley marvelled at its magical properties, capable of reigniting an ember of wood and increasing the survival of mice in a closed container although the luckless Carl Wilhelm Scheele (1742–1786) had produced the gas ("fire-air") earlier and Antoine Laurent Lavoisier (1743– 1794) provided a more informed description of the true nature of O_2 naming it "oxigene" that had eluded Priestley who remained wedded to the "phlogiston theory"(West, 2014). But before we consider how the brain senses the "elixir of life" and the

neuroprotective mechanisms that collectively serve to preserve homeostasis when faced by the challenge of O_2 lack (hypoxia), it is important to remind ourselves that our fundamental need for O_2 obscures the fact that it is a toxic, mutagenic gas; deadly to the central nervous system (CNS) when in excess, yet paradoxically capable of sustaining life in physiologically controlled amounts.

Unlike the majority of stable molecules with all of their electrons housed as "spin opposed" pairs conforming with the Pauli Exclusion Principle (+½ + -½ denoted as ↑↓) (Figure 3A, upper left insert), a closer examination of its molecular orbital structure, reveals that triplet ground state (most stable) diatomic O_2 molecule ($^3\sum g^- O_2$) exists in air as a free (di)radical (Figure 3A) (Bailey, 2003; Bailey *et al.*, 2009). Technically speaking, we should refer to this gas as $O_2^{\bullet}$ [superscript dot denotes (2) unpaired electron(s)] and not simply O_2 since we're choosing to ignore what is arguably its most fascinating attribute! A lone electron is located in separate π^*_{2p} antibonding orbitals with the same spin quantum or spin states (+½ or ↑↑) consistent with Hund's rule (Hund, 1925). This molecular peculiarity renders O_2 paramagnetic allowing it to respond to a magnetic field, a property routinely exploited in numerous medical devices including oximeters, near infra-red spectrometers, magnetic resonance imaging and laboratory demonstrations whereby liquid O_2 is able to hang "suspended" when poured between the poles of a magnet (Figure 3A, upper right insert).

When O_2 attempts to oxidise another atom or molecule by accepting a spin opposed pair of electrons from it (↑**↓**), one of the electrons in the pair with a spin state opposite to that of the unpaired electron in O_2 would "fit" comfortably into the orbital, to create a spin-opposed pair (↑**↓**, bold arrow denotes the accepted electron). However, this would not be the case with the other electron given its parallel spin state (↑**↑**), thus preventing it from "pairing up" in accordance with the Pauli Exclusion Principle. Thus, unlike most other oxidising free radical species, this parallel spin renders O_2 less reactive at ''normal'' concentrations despite its powerful oxidising nature (Halliwell &

Gutteridge, 1984; Fridovich, 2013). This ''spin-restriction'' forces O_2 to accept its electrons one at a time, a thermodynamic quirk of fate that protects the C–H bonds of the brain's organic biomolecules from spontaneous combustion (Bailey *et al.*, 2009). It is the unusual combination of strong π bonding (remarkably high resonance stabilization energy of 100 kcal/mol) and weak σ bonding in $^{\bullet}OO^{\bullet}$ that enables this unique molecule to be abundant in Earth's atmosphere and provide the chemical energy to sustain aerobic life (Borden *et al.*, 2017), safely!

Janus face of O_2; too much of a good thing can kill you

Paradoxically however, this gas and products of its metabolism becomes toxic at elevated PO_2's, an original observation credited to Priestley who noted that a candle burned out faster in O_2 than in air, speculating that we humans may "...live out too fast, and the animal powers be too soon exhausted in this pure kind of air. A moralist, at least, may say, that the air which nature has provided for us is as good as we deserve" (Priestley, 1776). Further elaborations were provided by Paul Bert (1833-1886) who, in 1878, described convulsions in larks when exposed to 15-20 atmospheres, a response that subsequently became known as the "Bert Effect" (Bert, 1943). In modern times, supplemental O_2 (hyperoxia) is commonly used as part of the therapy of many circulatory disorders yet it is well known that the gas can exert toxic effects when not used judiciously, damaging the CNS, eyes and lungs.

However, it wasn't until 1954 that the damaging of effects of O_2 toxicity were eventually linked to free radical formation (Gerschman *et al.*, 1954), more specifically increased mitochondrial formation of the univalent reductant, the superoxide anion ($O_2^{-\bullet}$) (Chance *et al.*, 1979) (Figure 3B). Though not especially "super" [one electron reduction potential ($E^{O'}$) = +940 mV], $O_2^{-\bullet}$ can be converted to

hydrogen peroxide (H_2O_2) through reduction or dismutation and upon reaction with transition metal ions, ultimately forming the hydroxyl radical ($OH^{\bullet}$). This species is at the top of the free radical "league of reactivity" ($E^{O'}$ = +2310mV), thermodynamically capable of oxidising any biomolecule that it collides with at a rate constant very near the diffusion limit (Buettner, 1993).

Contemporary physiology has taught us the conceptual significance of the ''O_2-cascade'', highlighting that the ever-decreasing PO_2 gradient serves to provide a ''pressure-head'' to maintain diffusive O_2 flux driving the gas from the capillary into the (cerebral) mitochondrion (Wagner, 1996). But perhaps we need to consider an alternative viewpoint; the endogenous resistances offered to O_2 transport (i.e. the sequential, progressive reduction in PO_2) may have evolved as an alternative form of endogenous antioxidant defence, limiting the concentration of (toxic) O_2 to which the mitochondrion is exposed (P_{50} for PO_2-dependant mitochondrial O_2 consumption < 1 mmHg), given its inherent vulnerability to oxidative damage and corresponding respiratory dysfunction (Hill et al., 2018). The fact that the Michaelis constant (K_m) of the terminal reductant, cytochrome c oxidase, for O_2 is so extraordinarily low (0.03–0.3 mmHg) (Vanderkooi *et al.*, 1991) stands testament to how important it is to harness this molecule and maintain cellular PO_2 within ''safe'' manageable physiological limits. Indeed, increasing O_2 levels equivalent to the conditions typically encountered in most isolated mitochondrial studies amplifies uncoupled mitochondrial proton leak and oxidative stress reducing bioenergetic efficiency (Gnaiger *et al.*, 2000).

The brain and oxidative stress; bittersweet balance

Our reliance on this toxic gas is matched by an equally fascinating fact in that despite its limited regenerative capacity, the brain is poorly equipped to cope with these potentially damaging O_2-

induced free radical reactions. Nervous tissue seems to out-perform other tissues in that it is capable of generating more $O_2^{-\bullet}$ with antioxidant defenses that are modest at best and neuronal membrane lipids rich in eicosapentaenoic (C20:5) and docosahexaenoic (C22:6) polyunsaturated fatty-acid side chains are especially susceptible to lipid peroxidation (Bailey, 2003; Bailey *et al.*, 2009; Cobley *et al.*, 2018).

Indeed, second to adipose tissue, nerve tissue contains the highest concentration of these highly peroxidisable lipids. Furthermore, a dense network of mitochondria exposed to high mass-specific O_2 flux, an abundance of autoxidisable neurotransmitters, cytochrome P450 and reactive microglia also serve to compound $O_2^{-\bullet}$ formation. Excitotoxic amino acids, highly active neuronal Ca^{2+} trafficking, excessive glucose/glutamate uptake and enrichment of redox-active transition metals with the capacity to catalyse Fenton/Haber–Weiss-driven generation of $OH^{\bullet}$ to initiate neuronal apoptosis and further compound membrane destabilization and vascular damage further contribute to the brain's 'oxidant burden sensitizing it's potential to damage (Bailey, 2003; Bailey *et al.*, 2009; Cobley *et al.*, 2018). This, however, is not as much of a paradox as was once thought (see later).

Given that its O_2 supply is so delicate coupled with its limited ability to contain these potentially damaging free radical chain reactions, it would seem intuitive for evolution to favor feedback/forward mechanisms capable of sensing subtle changes in hypoxia and orchestrating transmission of signals to the cerebrovasculature coupling local cerebral O_2 delivery (CDO_2) to tissue metabolic demand such that cerebral homeostasis remains preserved consistent with the conservation of mass principle (Bailey *et al.*, 2017b). Indeed, evolution appears to have perfected this millions of years ago with the emergence of anoxia-tolerant vertebrates such as the freshwater turtle (*Trachemys scripta and Chrysemys picta*) and the crucian carp (*Carassius carassius*). These species can negotiate brain survival through specialisations of brain physiology despite days to

weeks of anoxia, entering into a state of deep hypometabolism and suppression of cellular injury during anoxia-reoxygenation (Nilsson & Lutz, 2004; Larson *et al.*, 2014).

Cerebral O_2 sensing

Given the evolutionary importance of O_2 for the maintenance of complex life, it is likely that the ability to "sense" subtle changes in PO_2 and mount a defence against metabolic compromise and/or structural damage was one of the first roles of the CNS and likely represented a major driving force in the evolution of the human brain, thus providing a selective advantage (Costa *et al.*, 2014). Indeed, the CNS regulates neural activity of the cardiovascular and respiratory systems that are located almost exclusively in the brainstem, one the most primitive neuroanatomical regions of the human brain (~ 300 Mya) that has remained highly conserved across vertebrate evolution (Northcutt, 2002). It is becoming increasingly clear that an inability to sense O_2 adequately and failure to orchestrate coordinated transmission of vasoactive signals to the cerebrovasculature uncoupling local cerebral O_2 delivery to tissue metabolic demand has been implicated in the pathophysiology of a variety of CNS disorders including stroke, head trauma, neoplasia, vascular malformations and neurodegenerative diseases, highlighting its clinical importance (Sharp & Bernaudin, 2004).

Systemic hypoxia is acutely sensed by central (carotid body) and peripheral (pulmonary arteries, ductus arteriosus, adrenal medulla, neuroepithelial bodies in the lung) chemoreceptors that initiate cardiorespiratory reflexes that collectively serve to improve pulmonary gas exchange and cerebral O_2 delivery (Sharp & Bernaudin, 2004; Weir *et al.*, 2005). A key regulatory role has been assigned to the red blood cell including its ability to autonomously regulate its own deformability and flow

velocity through capillaries (Wei *et al.*, 2016) with haemoglobin implicated as the hypoxic sensor capable of releasing vasoactive metabolites from neurons, astrocytes, pericytes and smooth muscle cells (Singel & Stamler, 2005). While numerous mediators including *β*-adrenergic receptor activation, prostaglandins, epoxyeicosatrienoic acids, ATP-sensitive potassium channels, adenosine, free radicals and associated reactive oxygen/nitrogen species have been proposed, considerable evidence supports an increasingly important role for nitric oxide with the stable metabolites nitrite and *S*-nitrosohaemoglobin widely contested given their ability to conserve and transfer bioactivity within the microcirculation (Stamler *et al.*, 1997; Cosby *et al.*, 2003; Bailey *et al.*, 2017a).

Longer term adjustments are achieved through differential regulation of the highly conserved transcriptional complex hypoxia-inducible factor (HIF), whose complexity has increased in tandem with the evolution of ever-more sophisticated O_2 transport systems and rising atmospheric O_2 levels (Taylor & McElwain, 2010) (Figure 4A). Members of the HIF gene family encode both alpha (*α*) and beta (*β*) subunits that form functional heterodimers to regulate transcription (Wang *et al.*, 1995). In humans there are three paralogs of the HIF-*α* subunit (HIF-1*α*, HIF-2*α*/EPAS, HIF-3*α*) and two paralogs of the HIF-*β* subunit (ARNT, ARNT2).

In normoxia, HIF-1*α* is oxidised (hydroxylated) by prolyl hydroxylases (PHDs) using *α*-ketoglutarate. The hydroxylated HIF-1*α* subunit interacts with the von Hippel–Lindau protein and is subsequently catabolised by proteasomes, such that HIF-1*α* is continuously synthesized and degraded. However, in hypoxia, HIF-1*α* hydroxylation does not occur, stabilizing HIF-1 where it binds to a hypoxia response element leading to the expression of a wide variety of genes involved in angiogenesis, cell proliferation, erythropoiesis, glucose transport, glycolytic metabolism and cell survival (Semenza, 2007; Ratcliffe, 2013).

While recent advances have revealed the molecular underpinnings of this highly conserved pathway, a hotly debated topic relates to the precise molecular identity of the O_2 sensor (Kemp, 2006). While numerous models have been proposed (Neubauer & Sunderram, 2004), accumulating evidence suggests a central role for the mitochondrion which makes intuitive sense given its intimate relationship with O_2 and fact that cytochrome aa3 represents its terminal acceptor. More specifically, it has been suggested that hypoxia increases $O_2^{-\bullet}$ formation from Complex III of the electron transport chain possibly by increasing ubisemiquinone lifetime at the outer ubiquinone binding site (Qo) with release to the intermembrane space and subsequent hydrogen peroxide formation triggering HIF-*α* stabilisation subsequent to PHD inactivation potentially related to phosphorylation or decreased bioavailability of Fe (II) (Chandel *et al.*, 1998; Bell *et al.*, 2007; Smith *et al.*, 2017) (Figure 4B). However, this theory is not without its critics and remains a source of ongoing debate (Ward, 2006; Weir & Archer, 2006), considered by some as controversial if not indeed counterintuitive ($\downarrow O_2 \rightarrow \downarrow$electron flux/uncoupled leakage) with evidence supporting a more direct link between molecular O_2 and PHD inhibition/HIF activation (Dunham-Snary *et al.*, 2016).

Importantly however, the ability to respond to subtle changes in ambient oxygenation using $O_2^{-\bullet}$ as an ancient signal transductant in addition to protection against oxidative stress was present even in the last universal common ancestor (LUCA), a genetically and metabolically diverse community containing the molecular origins of all present life forms estimated to have appeared ~3.8 Gya (Slesak *et al.*, 2012; Briehl, 2015). Combined with the emerging concept of oxidative hormesis, it is becoming increasingly clear that at physiological concentrations, free radicals and associated reactive oxygen species (ROS) have the adaptive capacity to preserve cerebral O_2 homeostasis through cell-cell communication and should not simply be constrained to toxic, mutagenic

"accidents" of in-vivo chemistry limited to cellular oxidative damage and pathophysiology (Bailey *et al.*, 2018).

Lessons from another O_2 paradox; neurovascular coupling

It has been suggested that maintaining a relatively high PcO_2 (~25 mmHg) in the face of increased $CMRO_2$ can explain the puzzling mismatch of the larger fractional increase in CBF (by a factor of 2-3) that accompanies a modest increase in $CMRO_2$ (Buxton, 2010; Devor *et al.*, 2011) a phenomenon originally coined "focal physiological uncoupling" (Fox & Raichle, 1986). This unexpected discrepancy between flow and metabolism forms the basis for current blood-O_2-level-dependent functional magnetic resonance imaging methods (Kwong *et al.*, 1992; Ogawa *et al.*, 1992) and may have evolved to provide a safety margin for CDO_2, preventing a fall in PcO_2 (Buxton, 2010; Devor *et al.*, 2011; Leithner & Royl, 2014). The need to maintain PcO_2 so high appears counterintuitive given such a low K_m for cytochrome oxidase (< 1 mmHg) but may be required due to other competing reactions that also require O_2 as a substrate yet exhibit considerably higher K_m's (Erecinska & Silver, 2001) and need to maintain the cellular phosphorylation ratio ([ATP]/[ADP][Pi]) that begins to decrease at considerably higher PO_2's (P_{50} ~12 mmHg) (Wilson, 2015).

However, if it's true that PcO_2 needs to be maintained so high in the brain, what drives the functional hyperaemia when neural activity and $CMRO_2$ increase? It would seem unlikely to be a reduction in PcO_2 detected by an O_2 sensor given the brain's limited O_2 reserves (1 s) and fractional extraction (50 %) combined with the temporal delay of a few seconds before CBF increases; the PcO_2-mediated feedback signal would be dangerously slow. For this reason, neurovascular coupling specialists argue that the immediate trigger for the CBF increase is probably not driven by an O_2 sensor, but rather by more direct feed-forward mechanisms related to the increased neural activity

itself that is subsequently modulated by (slower) feedback responses (Buxton, 2010). Candidate mechanisms include neurotransmitters, nitric oxide, extracellular potassium and arachidonic acid metabolites (Attwell & Iadecola, 2002; Hamel, 2006) notwithstanding signaling changes from the neurovascular unit itself (Attwell *et al.*, 2010) though we cannot discount potential contributions from (other) free radicals and associated ROS/reactive nitrogen species (RNS) in light of their high reactivity and short half-lives (Bailey *et al.*, 2018). In short, the lack of a buffer of O_2 means evolution has had to develop multiple (safer) feed-forward mechanisms related to neural activity to drive the increase in perfusion, in anticipation of the upcoming need for increased CDO_2 to preserve PcO_2.

Quantum redox signalling; an emerging concept

Since the brain's evolution and ongoing survival depends on its constancy of electron flow, it would be remiss not to make albeit brief reference to quantum neuroscience, an emerging discipline focused at the biological quantum/classical interface, that promises to offer unique insight into the finer details of O_2 sensing that classical approaches otherwise fail to explain. Erwin Schrödinger (1887-1961) famous for his wave equation for non-relativistic quantum mechanics (QM) given by: $\hat{H}|\psi\rangle = E|\psi\rangle$ (time-independent equation where $\hat{H}$ = Hamiltonian operator, E = energy and ψ = wave function that describes velocity or location of a particle) was the first to ask if biological systems harness QM to perform a task more efficiently than even the best classical equivalent for selective advantage (Schrödinger, 1944). Initially met with fierce resistance given such seemingly counterintuitive concepts as (quantum) superposition (a particle can be in two places at once and exist in different states, both as a particle and a wave), entanglement (two particles at a distance form a relationship) and tunnelling (a particle can pass through a solid object) and challenges posed

by the impossibly warm, wet brain that collapses coherence (and hence QM effects), emerging evidence now suggests that there may well be some cases in which QM does indeed provide a biological advantage (Wolynes, 2009; Ball, 2011; Lambert *et al.*, 2013).

QM appears to be exploited by Nature during avian navigation, olfaction and arguably the best described of all, light harvesting in photosynthesis allowing excitons, generated by ancient green, sulphur-breathing bacteria, to travel as a coordinated wave rather than (classically) as a simple straight line, "feeling out" the most efficient pathway to transport energy to the plant's reaction centre within a staggeringly short, 10^{-9} second, achieving close to 100 % efficiency (Thyrhaug *et al.*, 2018). Could the mitochondrial formation of free radicals, themselves sub-atomic species, exploit quantum-based signalling to preserve cerebral O_2 homeostasis? Preliminary evidence suggests that this may well be the case with formation of "spin-correlated radical pairs" mediated by weak magnetic fields and evidence for mitochondrial electron tunnelling and entanglement (Usselman *et al.*, 2014; Nunn *et al.*, 2016; Usselman *et al.*, 2016) forcing a reappraisal of currently (i.e. classically) accepted concepts revealing more complex cellular and molecular mechanisms than previously thought (Figure 4B).

Conclusion

The current review has explored the intimate relationship between rising atmospheric O_2 levels and evolution of life on Earth and the brain, emphasising how challenging it is for the human brain to use O_2 safely and effectively, and some of the unexpected consequences of our dependence on O_2. The modern-day human has evolved with an oversized brain exquisitely vulnerable to failure given that it is entirely reliant on O_2, a toxic, mutagenic free radical gas that exists in air as a diradical, deadly in

excess yet paradoxically capable of sustaining life in controlled physiological amounts. By further exploring O_2's "quantum quirkiness", our understanding of precisely how the human brain senses hypoxia and the elaborate redox-signaling defence mechanisms that emerging evidence suggests may harness QM to preserve O_2 homeostasis has the potential to offer unique insights into the pathophysiology and treatment of human brain disease.

Abbreviations

ATP: Adenosine triphosphate; C: Carboniferous; CBP: Cyclic adenosine monophosphate-response element binding protein; CDO_2: Cerebral oxygen delivery; $CMRO_2$: Cerebral metabolic rate of oxygen; CNS: Central nervous system; cO_2: Cerebral oxygen content; D: Devonian; Є: Cambrian; *E*: energy; $E^{O'}$: One electron reduction potential; FIH: Factor inhibiting hypoxia-inducible factor; GOE: Great oxidation event; Gya: Billion years ago; $\widehat{H}|\psi$: Hamiltonian operator; H_2O_2: Hydrogen peroxide; HIF: Hypoxia-inducible factor; HIF-1$\alpha/_{\beta}$: Hypoxia-inducible factor alpha/beta; HRE: Hypoxia response element; J: Jurassic; K: Cretaceous; Km: Michaelis constant; LUCA: Last universal common ancestor; Mya: Million years ago; NOE: Neoproterozoic event; $O_2/O_2^{\bullet}$: Oxygen; $O_2^{-\bullet}$: Superoxide anion; $OH^{\bullet}$: Hydroxyl radical; $^3\sum g^- O_2$: Triplet ground state oxygen; O: Ordovician; PcO_2: Cerebral tissue partial pressure of oxygen; P_{50}: partial pressure of oxygen required to achieve 50% hemoglobin saturation; PHD: Prolyl hydroxylase; P: Permian; Pre-Є: Pre-Cambrian; ψ = wave function; S: Silurian; T: Tertiary; Tr: Triassic; VHL: Von Hippel Lindau tumor suppressor protein; QM: Quantum mechanics

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Competing interests

The author declares he has no competing interests.

Legends

Abstract. Summary of the intrinsic links between oxygen (O_2), evolution and human defence of cerebral homeostasis

Rising paleoatmospheric O_2 levels, especially during the past ~550-600 million years (Phanerozoic eon) has been linked to major evolutionary and developmental events including emergence of the mitochondrion and central nervous system. The human brain has since evolved to be entirely dependent on O_2 and as a consequence is especially vulnerable to failure. However, the "elixir of life" is Janus-faced, capable of sustaining life in physiologically controlled amounts yet paradoxically deadly when in excess, its toxicity and mutagenicity due to the fact that it exists in air as a free radical with two unpaired electrons located in separate antibonding orbitals. The mitochondrion is able to "sense" O_2 deprivation (hypoxia) orchestrating release of free radicals and associated reactive oxygen species (ROS) that serve as signal transductants capable of effecting neuroprotective adaptation through stabilisation of hypoxia-inducible factor alpha (HIF-1α) with gene transcription ultimately helping defend cerebral O_2 homeostasis. Emerging evidence suggests that mitochondria may harness quantum mechanics to sense O_2 more

efficiently than even the best classical equivalent for selective advantage revealing more complex cellular and molecular mechanisms than previously thought.

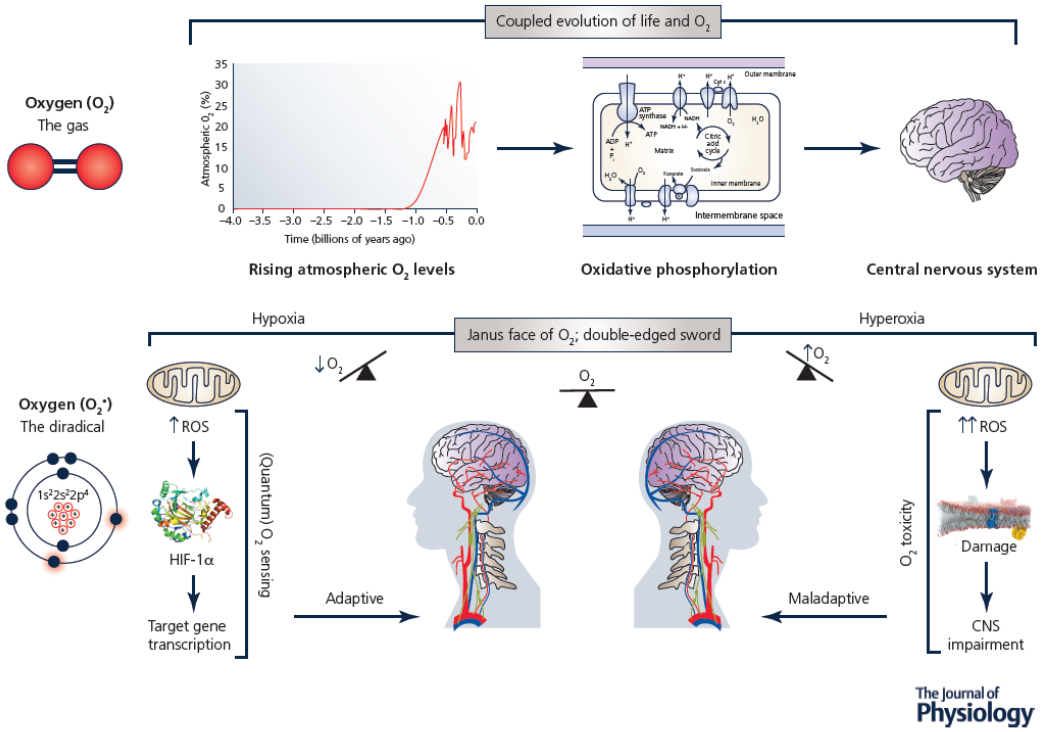


Figure 1. Coupled evolution of life and atmospheric oxygen

Major evolutionary and developmental events that have been linked to "pulses" in the atmospheric oxygen (O_2) concentration based on the GEOCARBSULPH model (Berner, 2007; Berner, 2009). **A.** Note that since the origin of life within 500 million years of Earth's formation (LUCA, Last Universal Common Ancestor), oxygenic photosynthesis was responsible for the rapid increase in atmospheric O_2 levels during the Proterozoic Eon of the Pre-Cambrian period (~0-10% in < 1 billion years) preceded by endosymbiosis, emergence of cellular respiration with adenosine triphosphate the universal energy source and cephalisation, a characteristic feature of the ancestral bilateria, leading to the first appearance of a central nervous system (Holland *et al.*, 2013). Earth's oxidation was likely paralleled by selective pressure favouring survival of organisms that could tolerate O_2 toxicity and control oxidative processes to harness energy including cellular protection through evolution of antioxidant defence though sequence analyses suggest that even LUCA was capable of detoxifying reactive oxygen species (ROS) long before O_2 became abundant in the atmosphere or ocean likely the result of localised O_2 formation via abiotic sources (e.g. photolysis of water by ultraviolet light) or cohabitation with an oxidative photosynthesising organism (Case, 2017). Three primary antioxidant enzymes arose prior to the GOE; superoxide dismutase, catalase and peroxiredoxins (Case, 2017). Pre-Є, Pre-Cambrian; Є, Cambrian; O, Ordovician; S, Silurian; D, Devonian; C, Carboniferous; P, Permian; Tr, Triassic; J, Jurassic; K, Cretaceous; T, Tertiary (Berner *et al.*, 2007). **B.** Stochastic changes in atmospheric O_2 levels during the Phanerozoic eon peaked during the Carboniferous/Permian periods resulting in gigantism subsequent to augmented O_2 diffusive capacity and heralded major evolutionary advances that included a 3.5-fold increase in hominin brain volume over ~2.75 million years (Seymour *et al.*, 2016). Also note the three major extinction events (red bands) associated with

dramatic falls in atmospheric O_2 levels. **C.** Parabolic projection of the decline in future atmospheric O_2 levels using a stochastic model (Livina *et al.*, 2015) applied to original data obtained from recording stations in the Scripps Programme (Keeling, 1988). Note that the model predicts that in ~3,600 years, atmospheric O_2 levels will be so low that hypoxia will be encountered even at sea-level, equivalent to being exposed to a terrestrial altitude of ~5,340 m which represents the highest elevation know to sustain lifelong human habitation with complete (O_2) depletion predicted within ~4.4 millennia (Martin *et al.*, 2017).

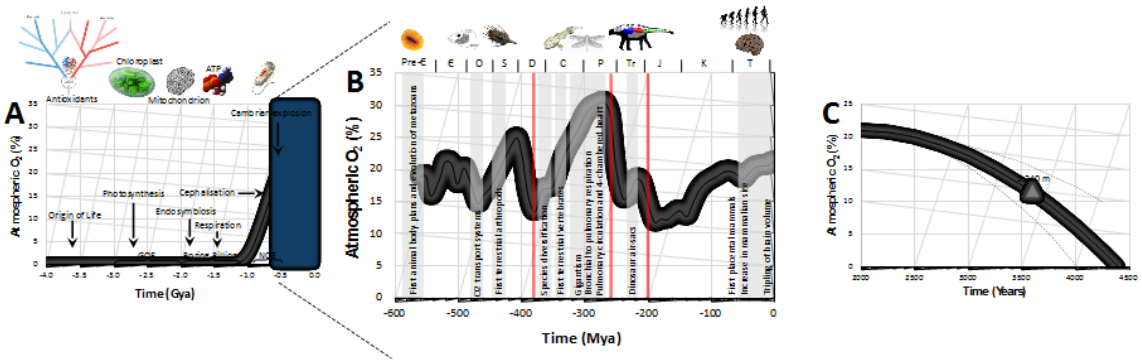


Figure 2. Evolution of the hominin brain and vulnerability to failure

A. Exponential increase in cranial capacity observed in fossil hominids over time beginning with Homo Habilis and marked encephalisation linked to the physically active "Hunter Gatherer", Homo Erectus (annotated). Data based on the (calculated) mean of published individual data points (Schoenemann, 2006). Note also the increase in total number of neurones estimated from separate derivations of cranial capacity and corresponding increases in cerebral blood flow calculated from the size of the internal carotid foramina, in relation to endocranial volume (Seymour *et al.*, 2016). **B.** The human brain's oxygen dependence comes at a cost with a corresponding high vulnerability to failure given that it is an entirely aerobic organ characterised by limited energy reserves that becomes evident when confronted by complete oxygen lack (anoxia). $CMRO_2$, cerebral metabolic rate of oxygen; PcO_2 (average) cerebral tissue partial pressure of O_2; cO_2, cerebral oxygen content.

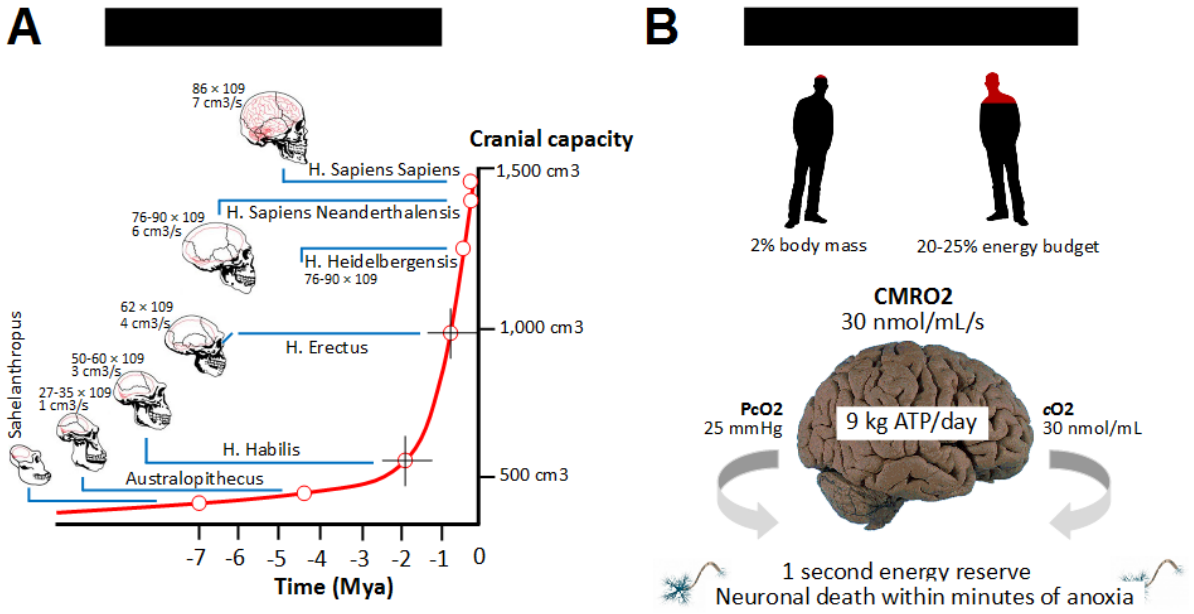


Figure 3. A. Molecular orbital diagram of the most stable form (electronic ground state) of the diatomic oxygen molecule ($^3\sum g^- O_2$) and B. Biological reactions underpinning oxygen toxicity in the central nervous system.

A. Each line represents a molecular orbital and the arrows represent electrons, the direction of which indicates their spin quantum number. Note that oxygen (O_2) with an electronic structure of $1s^2 2s^2 2p^4$ qualifies as a di-radical since it contains two unpaired electrons each occupying different π^*_{2p} anti-bonding orbitals (highlighted in red) with the same spin quantum number (parallel spin) in accordance with Hund's rule. It is for this reason that O_2 is paramagnetic allowing liquid O_2 to hang magically suspended between the poles of a magnet (upper right insert). During the process of oxidation when O_2 looks to accept a (spin opposed) pair of electrons ($\uparrow\downarrow$), only one of the pair ($\downarrow$) can "fit" into each of the vacant π^*_{2p} anti-bonding orbitals to create a spin opposed pair (as indicated). Hence, O_2 thermodynamically prefers to accept only one electron at a time to conform with the Pauli Exclusion Principle [named after the Nobel Prize winning work of the Austrian physicist Wolfgang Pauli (1900-1958), photograph upper left insert]. Fortuitously, this "spin restriction" means that O_2 reacts "sluggishly" with the brain's organic compounds with the organic donor having to undergo a "slow spin inversion'" to donate its electrons. **B.** Three types of reactions lead to the superoxide anion ($O_2^{-\bullet}$)-mediated formation of the damaging hydroxyl radical ($HO^\bullet$) capable of causing indiscriminate damage to biological cell membranes that characterises O_2 toxicity ; [1] one-electron reduction of molecular O_2 to $O_2^{-\bullet}$ catalysed by transition metals including iron (Fe), [2] Fenton reactions that involve metal-catalysed formation of $HO^\bullet$ and [3] Haber-Weiss reaction involving the combination of $O_2^{-\bullet}$ and hydrogen peroxide (H_2O_2) to yield additional $HO^\bullet$.

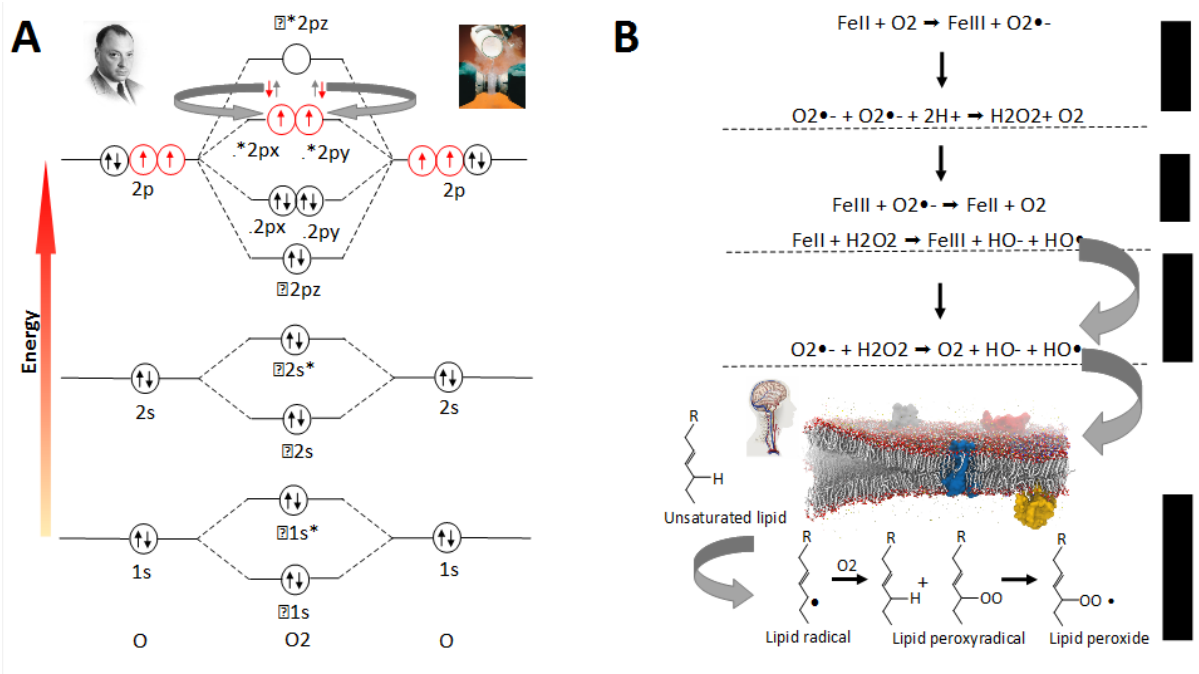
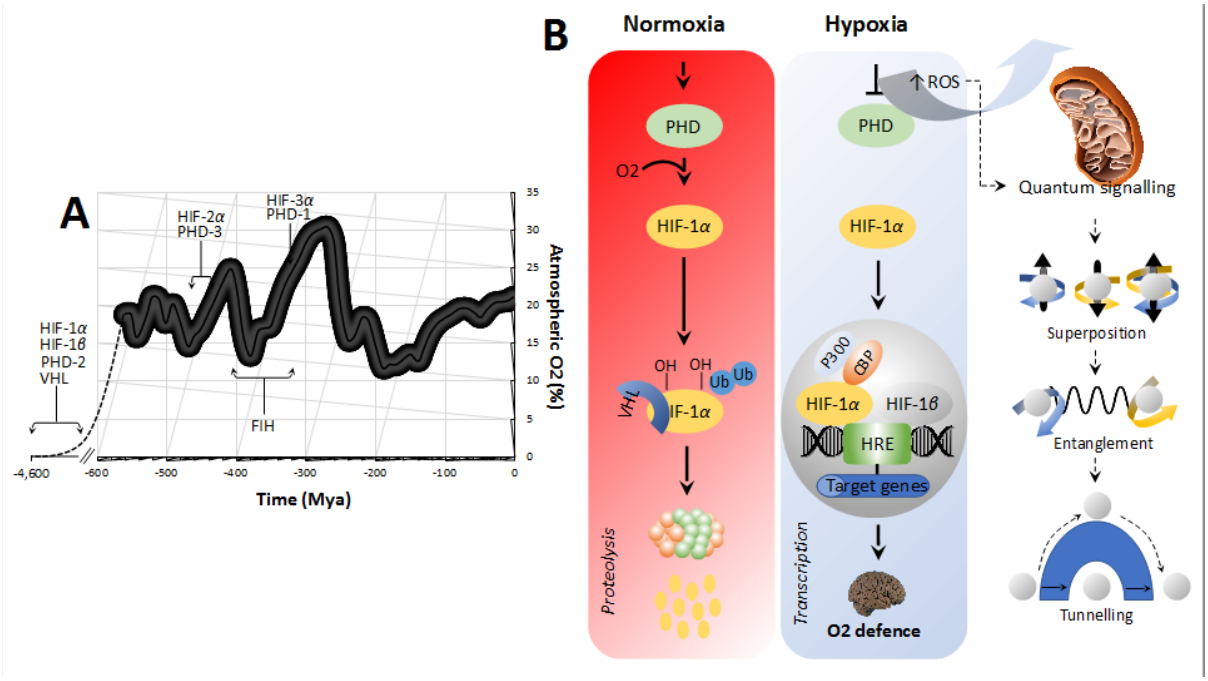


Figure 4. A. Evolution of genes encoding the hypoxia-inducible factor (HIF) pathway and B. Importance of mitochondrial-generated reactive oxygen species (ROS) stabilization of HIF-1*α* during hypoxia including emergent quantum signalling aspects.

A. Appearance of genes based on published approximations (Taylor & McElwain, 2010). **B.** During normoxia, hypoxia-inducible factor-1 alpha (HIF-1*α*) is hydroxylated on prolines by the prolyl hydroxylases (PHD), tagging it for recognition by the von Hippel Lindau tumor suppressor protein (VHL) resulting in the continual ubiquitination and degradation of HIF-1*α*. During hypoxia, the mitochondrial formation of the superoxide anion from the Qo site of the bc1 complex of Complex III are released into the intermembrane space and enter the cytosol to decrease PHD activity preventing hydroxylation resulting in HIF-1*α* stabilisation and transcription of genes that collectively preserve cerebral oxygen (O_2) homeostasis. Note that "quantum" aspects of cerebral O_2 sensing are also outlined. FIH, factor inhibiting HIF; CBP, cyclic AMP-response element binding protein; HRE, hypoxia response element.



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